

AMENDMENTS TO THE CLAIMS

Listing of Claims:

Claims 1-12 cancelled.

13. (currently amended) A method for the production of a purified recombinant invasin protein comprising:

- a) ~~inserting~~ preparing an expression vector comprising a polynucleotide encoding an invasin protein ~~into an expression vector~~;
- b) ~~transforming the combination of a)~~ into a host cell with the expression vector;
- c) growing the transformed host cell under conditions conducive to soluble invasin protein expression;
- d) extracting the expressed invasin protein from a ~~host cell~~ lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an reconstituted organism reconstituted from the transformed host cell with a solution comprising a protein denaturant;
- e) performing an affinity purification of the extracted invasin protein ~~wherein the method of said purification is performed in the presence of a~~ the protein denaturant;
- f) removing said protein denaturant from the purified invasin protein ~~solution obtained in the purification process of e)~~ until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein ~~solubility~~; and
- g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

14. (currently amended) A method for the production of a purified recombinant invasin protein comprising:

- a) ~~combining~~ preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety;
- b) ~~transforming the combination of a), in an appropriate expression vector,~~
into a host cell with the expression vector;
- c) growing the transformed host cell under conditions conducive to soluble invasin protein expression;
- d) extracting the expressed invasin protein from a ~~host cell lysate of the transformed host cell,~~ a culture medium comprising the transformed host cell, or an reconstituted organism reconstituted from the transformed host cell with a solution comprising a protein denaturant;
- e) performing an affinity purification of the extracted invasin protein ~~appropriate for the affinity purification moiety encoded by the polynucleotide in a), wherein the method of said purification is performed in the presence of a~~ the protein denaturant;
- f) removing said protein denaturant from the purified invasin protein ~~solution obtained in the purification process of e)~~ until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein ~~solubility~~; and
- g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

15. (original) The method of claim 14 wherein the affinity purification moiety is His-Tag.

16. (previously presented) The method of claim 14 wherein the protein denaturant is selected from the group consisting of guanidine hydrochloride, detergents, and urea.

17. (previously presented) The method of claim 14 wherein the protein denaturant is urea.

18. (original) The method of claim 17 wherein the concentration of urea is between about 1 M and about 10 M.

Claims 19-20 cancelled.

21. (currently amended) The method of claim 14 further comprising the step of removing the affinity purification moiety from the recombinant invasin protein after step e, f, or g.

22. (currently amended) The method of claim 14 wherein the dilution of the purified invasin protein occurs in about 1 minute or less.

Claims 23-24 cancelled.

25. (currently amended) A method for the production of a purified recombinant invasin protein comprising:

- a) ~~combining~~ preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety;
- b) ~~transforming the combination of a), in an appropriate expression vector,~~
into a host cell with the expression vector;

- c) growing the transformed host cell under conditions conducive to soluble invasin protein expression;
- d) extracting the expressed invasin protein from a ~~host cell~~ lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an reconstituted organism reconstituted from the transformed host cell with a solution comprising 6 M urea;
- e) performing an affinity purification of the extracted invasin protein ~~appropriate for the affinity purification moiety encoded by the polynucleotide in a), wherein the method of said purification is performed~~ in the presence of a protein denaturant;
- f) removing said protein denaturant from the purified invasin protein ~~solution obtained in the purification process of e)~~ until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein ~~solubility~~; and
- g) diluting the purified invasin protein in about 10 seconds or less into a volume of denaturant-free solution.

Claims 26-100 cancelled.

101. (previously presented) The method of claim 13 wherein the protein denaturant is selected from the group consisting of guanidine hydrochloride, detergents, and urea.

102. (previously presented) The method of claim 13 wherein the protein denaturant is urea.

103. (currently amended) The method of claim 13 wherein the dilution of the purified invasin protein occurs in about 1 minute or less.

104. (new) The method of claim 103 wherein the dilution of the purified invasin protein occurs in less than 10 seconds.

105. (new) The method of claim 13 wherein the purified invasin protein refolds without forming insoluble aggregates.

106. (new) The method of claim 13 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli*.

107. (new) The method of claim 106 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

108. (new) The method of claim 106 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

109. (new) The method of claim 13 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli* and the dilution of the purified invasin protein occurs in about 1 minute or less.

110. (new) The method of claim 109 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

111. (new) The method of claim 109 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

112. (new) The method of claim 14 wherein the purified invasin protein refolds without forming insoluble aggregates.

113. (new) The method of claim 14 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli*.

114. (new) The method of claim 113 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

115. (new) The method of claim 113 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

116. (new) The method of claim 14 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli* and the dilution of the purified invasin protein occurs in about 1 minute or less.

117. (new) The method of claim 116 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

118. (new) The method of claim 116 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

119. (new) The method of claim 22 wherein the dilution of the purified invasin protein occurs in less than 10 seconds.

120. (new) The method of claim 25 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli*.

121. (new) The method of claim 120 wherein the purified recombinant invasin protein is an IpaC or a SipC protein.

122. (new) The method of claim 120 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.